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An Examination of the Bacteriophages and Bacteria of the Namib Desert

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Bacteria and their viruses (called bacteriophages, or phages), have been found in virtually every ecological niche on Earth. Arid regions, including their most extreme form called deserts, represent the single largest ecosystem type on the Earth's terrestrial surface. The Namib desert is believed to be the oldest (80 million years) desert. We report here an initial analysis of bacteriophages isolated from the Namib desert using a combination of electron microscopy and genomic approaches. The virus-like particles observed by electron microscopy revealed 20 seemingly different phage-like morphologies and sizes belonging to the *Myoviridae* and *Siphoviridae* families of tailed phages. Pulsed-field gel electrophoresis revealed a majority of phage genomes of 55~65 kb in length, with genomes of approximately 200, 300, and 350 kb also observable. Sample sequencing of cloned phage DNA fragments revealed that approximately 50% appeared to be of bacterial origin. Of the remaining DNA sequences, approximately 50% displayed no significant match to any sequence in the databases. The majority of the 16S rDNA sequences amplified from DNA extracted from the sand displayed considerable (94~98%) homology to members of the Firmicutes, and in particular to members of the genus *Bacillus*, though members of the Bacteroidetes, Planctomycetes, Chloroflexi, and δ-Proteobacteria groups were also observed.

***Keywords*:** bacteriophages, desert sand, 16S rDNA, electron microscopy

Bacteria, and their viruses (called bacteriophages or phages), have been found in virtually every studied ecological niche on Earth, including those that were previously thought to be uninhabitable (Antranikian *et al.*, 2005). These latter ecosystems include deep sea thermal vents, the extreme cold biomes at the two poles (Vincent *et al*., 2000; Geslin *et al.*, 2003), and deserts (Nagy *et al.*, 2005; Prigent *et al.*, 2005; Chanal *et al.*, 2006). Arid regions, including their most extreme form called deserts, likely represent the single largest ecosystem on the earth's terrestrial surface. The recognition of their importance has been highlighted by the United Nation's declaration of the year 2006 as the International Year of Deserts and Desertification. Due to human activity and global warming, desert regions are increasing in size, with a concomitant increase in desert sand storms in Asia and Africa. The control of desertification, and the ability to reclaim certain desert regions, will require a complete knowledge of their characteristics, including their biology.

The ecology of deserts has usually been studied with respect to their macro fauna and macro flora, and an impressive amount of information has been accumulated concerning the adaptive strategies of these organisms to the extreme fluctuations of temperature, high ultraviolet radiation exposure, and water deficiency found in this type of environment (Satyanarayana *et al*., 2005). However, much less detailed knowledge of the microorganisms inhabiting arid regions, and more specifically deserts, is available (Gallardo, 1995; Chanal *et al.*, 2006). The two main reasons for this state of affairs is that the vast majority of bacteria are not cultivatable under laboratory conditions (Amann *et al.*, 1995), and that genomic approaches to study the total microbial population have only recently become available and applied to the low biomass sand of deserts (Chanal *et al.*, 2006). Studies on the presence of bacteria, using both cultivation and 16S rDNA amplification and sequencing, have demonstrated that even the most severely dry and isolated desert regions contain a significant microbial population. These include members of both the Eubacterial and Archaeal kingdoms (Garcia-Pichel *et al.*, 2001; Dunbar *et al.*, 2002; Chanal *et al.*, 2006). The mechanisms underlying bacterial survival in desert conditions have yet to be extensively examined, though the majority of microorganisms on Earth are found to be embedded within biofilms (Donlan, 2002) consisting of exopolymeric substances that can afford protection from harsh environmental conditions (e.g. solar radiation, desiccation, extremes of pH, etc.) (Elasri and Miller, 1999; Davey and O'Toole, 2000). It is thus not totally surprising that a variety of bacteria were previously identified, through 16S rDNA analyses and cultivation under laboratory conditions, from the surface sands of the Sahara desert (Heulin *et al*., 2003; Chanal *et al*., 2006).

Most prior studies of bacteriophages in natural environments have focused on their characterization from aquatic ecosystems, or from relatively humid soils and sediments (Breitbart *et al.*, 2002; Rohwer, 2003; Williamson *et al.*, 2005). These studies have demonstrated that bacteriophages can

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be present in natural environments as extracellular virions, as quiescent prophage genomes, or as intracellular phage particles within so-called "pseudolysogens" (Ripp and Miller, 1997; Mann, 2003; Peduzzi and Schiemer, 2004). Bacteriophages have recently been shown to be present in the surface sands of the Sahara desert from Morocco and Tunisia (Prigent *et al.*, 2005). The three major morphotypes of tailed bacteriophages (Myoviridae, Siphoviridae, Podoviridae) were the most common types observed. The mechanisms by which these bacteriophages, with their potentially delicate tail structures, can survive in severe desert environments are not currently known.

We report here an exploratory analysis of bacteriophages isolated from the Namib desert using a combination of electron microscopy and genomic approaches. The Namib desert is believed to be the oldest desert on Earth, with an estimated age of over 80 million years, and is located in the southwestern tip of Africa. Though it stretches for over 1,500 km in length, it is only approximately 100 km wide. The inner desert regions are extremely dry, with an average annual rainfall of 1∼5 cm per year (Burke, 2001). Surface sand samples from three different locations of the desert were obtained and bacteriophage-like particles isolated from these samples were examined by electron microscopy. Pilot genomic libraries were prepared from the partially-purified bacteriophages, followed by sample sequencing, in order to further illustrate the bacteriophage populations. Moreover, 16S rDNA libraries were prepared from total DNA isolated from Namib desert sand, with Firmicutes, in particular members of the genus *Bacillus*, being found to be the most abundant bacterial group identified through sequencing of randomly-selected 16S rDNA clones. The bacteriophages and bacteria found in the Namib desert suggests that the study of phage and bacterial capacities to withstand extremes of temperature, desiccation, and exposure to ultraviolet radiation may provide important clues for the maintenance of life in this extremely rigorous environment.

Materials and Methods

Namib desert sample

Samples of surface sands were collected at 3 different locations: labelled NA A, NA B, and NA C, from the Namib desert (Fig. 1). A sample of the surface sand from each site was scooped into a sterile, 50 ml conical plastic centrifuge tube (a kind gift of T. Heulin, CEA Cadarache, France). Samples were stored in the dark at room temperature until use.

Isolation of bacteriophage-like particles

Five grams of sand from each sample were resuspended in 10 ml 1/5 LB media [Lennox L: 1% tryptone, 0.5% yeast extract, 1% NaCl, NaOH (3×10^{-3} M)] or 10 ml 1/4 TS media (Tryptic Soy broth, Difco), then sonicated on ice and incubated at 30°C for several hours with gentle shaking (Prigent *et al.*, 2005). Supernatant fluids were then collected after gravity sedimentation of the sand particles, and completed to 10 ml with the same media. Half of the sample was then incubated, after addition of mitomycin C to a final concentration of 1 μg/ml, for 20 min at 30°C with gentle shaking. The cells were washed twice by centrifugation at 6,000×g for 10 min at 4°C, resuspended in 10 ml of culture media to remove the mitomycin C, and incubated for 2.5 h at 30°C with gentle shaking. The supernatant fluids were collected after centrifugation (as above) and filtered through a 0.22 μm pore-size nylon filter. The bacteriophages were collected by ultracentrifugation at 100,000×g for 3 h at 4°C (Beckman TL-100 ultracentrifuge, TLS 100.4 rotor) and overlayed with 1× Mu buffer [20 mM Tris-HCl; pH 7.5, 200 mM NaCl, 20 mM $MgSO_4$, 1 mM $CaCl_2$, 0.1% (w/v) gelatin] at 4°C overnight. The bacteriophage pellet was then gently resuspended and stored at 4°C.

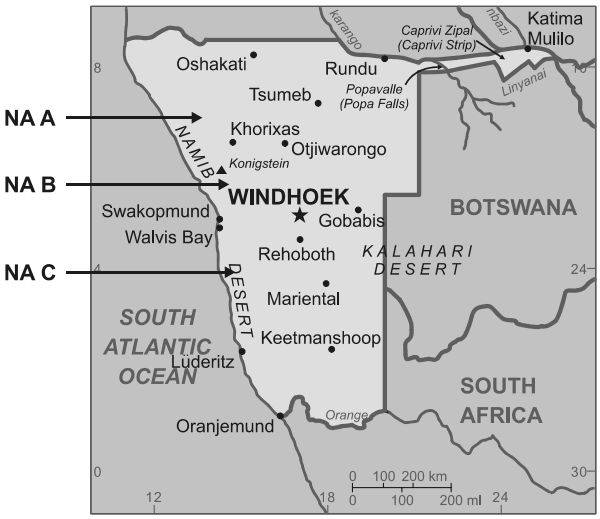

Fig. 1. The location of the sampling sites from the Namib desert. The arrows represent the location of sites where surface sand was sampled (GPS coordinates: NA A, S 19°57'675" E 13°02'960"; NA B, S 20°21'501" E 13°18'329"; NA C, S 22°51'387" E 11°35'044").

Electron microscopy

Formvar, carbon-coated copper electron microscopy grids (400 mesh) were overlaid with 10 μl of the bacteriophage suspensions for 5 min, and the attached phage particles negatively stained with 1% (w/v) phosphotungstenic acid for 1 min. Excess stain was removed and the grids allowed to air dry prior to examination using an EM 205 Philips electron microscope (microscope accelerating voltage 80 kV). Examples of phage particle morphologies observed at least ten times on different grids were photographed for further examination.

Bacteriophage genome diversity and size estimation

The nucleic acids of the bacteriophage suspensions (approximately 50 μl) were extracted from the virions with 50 μl phage extraction buffer [50 mM Tris-HCl; pH 7.5, 2 mM EDTA, 1% (w/v) SDS] for 10 min at 65°C followed by extraction in buffer-saturated phenol (pH 8) and ether extraction and subsequent ethanol precipitation. A fraction of the purified phage nucleic acids were then loaded onto a 1% agarose gel and subjected to pulsed field gel electrophoresis in 0.5× Tris-acetate-EDTA (TAE) buffer (20 mM Tris-acetate; pH 8.0, 5 mM EDTA) for 20 h at 14°C using a Bio-

Rad CHEF DR2 pulsed field gel electrophoresis apparatus at 4.5 V/cm, 120°, ramping at 10~100s linear slope. DNA was visualized under UV illumination after ethidium bromide staining.

Linker amplification shotgun library

A linker amplified shotgun library was created from the Namib viral community. The LASL/LL-PCR construction was adapted from the procedures of Nagesha *et al.* (1996) and Breitbart *et al.* (2002). Basically, 200 ng of the extracted bacteriophage nucleic acids were sonicated on ice for 16 sec at 33% duty cycle and maximum output 4 using a microprobe on a Branson model 450 Sonifier to obtain fragment sizes between 600 bp to 2,000 bp. The DNA fragments were then end-repaired using 3 units T4 DNA polymerase (Fermentas), 5 μg BSA (Fermentas), 0.5 μM dNTP mix (Fermentas), 1× T4 DNA polymerase buffer (Fermentas) for 30 min at 12°C. After repair, the DNA fragments were purified by extraction through buffer-saturated phenol (pH 8), followed by ether extraction and ethanol precipitation. Then, 1.25 μM of an asymmetric dsDNA *Not*1 linker (Proligo) were ligated to the blunt-ended DNA fragments using 5 units of T4 DNA ligase (Fermentas) in 1× T4 DNA ligase buffer (Fermentas). The sequence of the dsDNA linker is; 5'-CGTCAGTTAGCTAGC-3', with the primer sequence used for the subsequent PCR amplification underlined and the *Not*1 site in bold; 3'-GCAGTCAATCGAT**CGCCGGCG**ATCG ACTCATTCG-5'. The DNA fragments were subsequently purified by phenol extraction followed by ether extraction and ethanol precipitation and then amplified in a PCR reaction containing 1.2 μM linker primer (Proligo), 0.4 μM dNTP mix (Fermentas), 1× Thermopol buffer and 2 units High Fidelity PCR Enzyme mix (Fermentas). The conditions of amplification were: 2 min at 66°C, 2 min at 72°C, 2 min at 95°C followed by 30 cycles performed for 1 min at 95°C, 30 sec at 55°C, 2 min at 72°C, and a final elongation step for 7 min at 72°C. The amplified fragments were purified using a MinElute PCR Purification Kit (QIAGEN) and ligated into *Bse*JI (Fermentas)-digested plasmid pACYC184 and transformed by heat shock into *Escherichia coli* DH1 competent cells.

Analysis of DNA sequences

Sixty four clones from the Namib desert sand phage library (472 in total) were sequenced by Genome Express using pACYC184 primers (Proligo) flanking the unique *Bse*JI restriction site. These sequences were compared against those deposited in GenBank using TBLASTX and the e-values obtained used to classify the relative significance of sequences for their categorization as phage, bacteria, or unknown (Breitbart *et al.*, 2002).

Total DNA extraction

The procedure to extract the total DNA from the sand was adapted from Zhou *et al.* (1996). Five grams of sand were resuspended in 13.5 ml extraction buffer [100 mM Tris-HCl; pH 8, 100 mM Na EDTA; pH 8, 100 mM Na_2HPO_4, 1.5 M NaCl, 1% (w/v) CTAB] and then 74 μg/ml (final concentration) Pronase was added, followed by incubation at 37°C for 2 h with shaking. Then, 1.5 ml of 20% (w/v) SDS was added and incubation continued at 65°C for another 2 h. The mixture was then subjected to centrifugation at 6,000×g for 10 min at room temperature in a Heraeus Megafuge 10R centrifuge and the supernatant fluid was decanted. The pellet was extracted two more times with 4.5 ml extraction buffer plus 2% (w/v) SDS, mixed by vortexing for 10 sec and followed by incubation at 65°C for 10 min. The nucleic acids were extracted by the addition of an equal volume of chloroform/isoamyl alcohol (24:1), and precipitated using 0.6 volumes of isopropanol following a 1 h incubation at room temperature and centrifugation at 16,000×g for 20 min at room temperature in a Sorval RC-5C centrifuge. The DNA pellet was washed with 70% ethanol, followed by centrifugation at 16,000×g for 5 min at room temperature. The pellets were resuspended in 1/10× TE (1 mM Tris-HCl; pH 8, 0.1 mM Na EDTA; pH 8) at 4°C overnight and stored at -20°C until use.

16S rRNA gene library

The 16S rDNA sequences were amplified from the sand-extracted DNA using the universal primers 8F and 1492R (Crump *et al*., 1999). The 16S rDNA fragments were amplified in a 50 μl reaction using 2 μM primer (Operon), 0.4 μM dNTP mix (Fermentas), 1× Thermopol buffer (Fermentas), and 2 units High Fidelity PCR Enzyme mix (Fermentas). The conditions of amplification were: 5 min at 95°C followed by 30 cycles for 1 min at 95°C, 30 sec at 51°C, 2 min at 72°C, and a final elongation step for 7 min at 72°C. The amplified fragments were purified using a MinElute PCR Purification Kit (QIAGEN), ligated into pSmart LCKan (Lucigen), and transformed into *E. coli* DH10B competent cells. Selected clones from the 16S rDNA library were completely sequenced on both strands by Genome Express. The 16S rDNA sequences obtained were compared against the GenBank, RDB 2, Greengenes, and ssurRNA databases using BLAST.

The nucleotide sequences determined in this study have been deposited in the NCBI database under accession numbers EF636829 to EF636887.

Results

Virus-like particle (VLP) morphologies

Surface sand samples from three different locations of the Namib desert (Fig. 1) were examined for their bacteriophage populations. Based on structural criteria (Ackermann and DuBow, 2000), the VLPs observed by electron microscopy revealed at least 20 seemingly different phage-like morphologies and sizes (Fig. 2). The VLPs depicted here likely represent the most abundant active phage types present in the samples, and each VLP represents an example of a phage morphotype observed independently at least 10 times on the grids (Table 1). The measured dimensions of each of the displayed VLP morphotypes were found to vary by 10% or less. The observable VLP morphotypes had long non-contractile or contractile tails, and were thus assigned to the *Siphoviridae* or *Myoviridae* families, respectively (Fig. 2).

The *Myoviridae*-type bacteriophage particle types (Fig. 2H~S) were composed of non-enveloped capsids, separated by a neck-like structure, and a complex tail with a central tube and contractile sheath, generally ending in a base

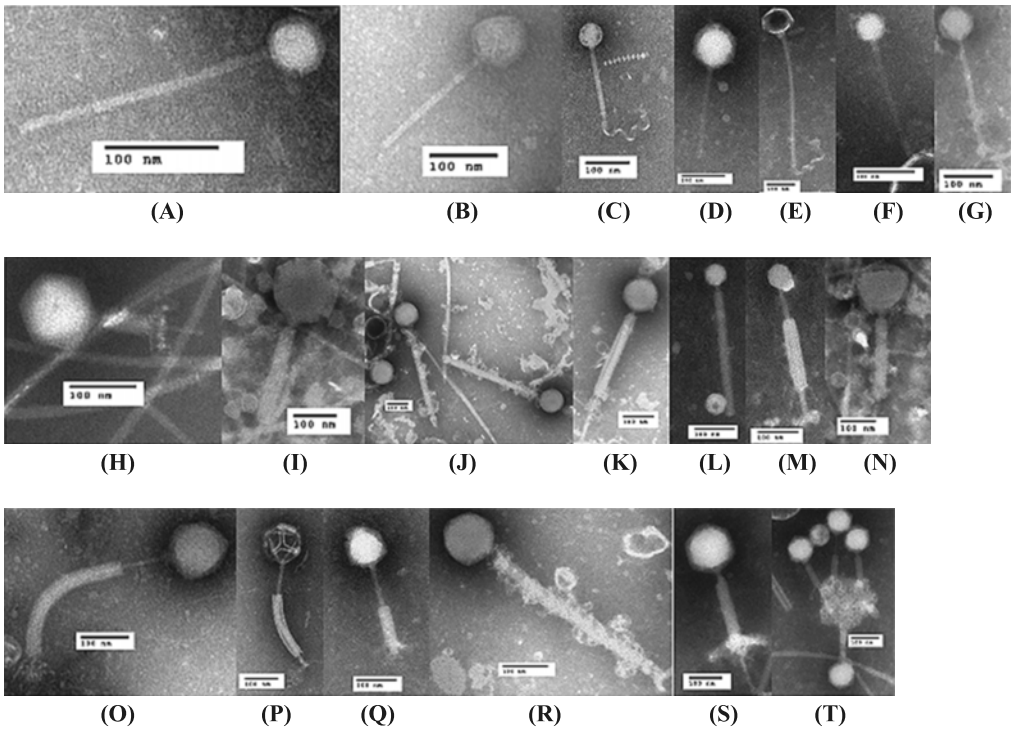

Fig. 2. Examples of phage particles isolated from the Namib desert samples. Phage particles were isolated as described in the 'Materials and Methods' section and examined with a Philips 205 electron microscope on Formvar carbon-coated copper grids after negative staining with phosphotungstate. Each photo contains a 100 nm scale bar.

Table 1. Virus-like particle morphotypes observed by electron microscopy[a]

Photo from Fig. 2	NA A[b]				NA B[b]				NA C[b]			
	LB+	LB	1/4TS+	1/4TS	LB+	LB	1/4TS+	1/4TS	LB+	LB	1/4TS+	1/4TS
A	-	-	++	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	+	-	-	-	-
C	-	-	-	-	-	-	+	+	-	-	-	-
D	-	+	+	-	-	-	-	-	-	-	-	-
E	-	+	-	+	-	-	-	-	-	-	-	-
F	-	-	-	-	-	-	-	-	-	-	-	+
G	-	-	-	-	-	-	-	-	-	-	+	-
H	-	+++	-	-	-	-	-	-	-	-	-	-
I	+++	-	-	-	-	-	-	-	-	-	-	-
J	-	-	-	++	-	-	-	-	-	-	-	+
K	-	-	++	+	-	-	-	-	-	-	+	+
L[c]	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++
M[c]	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++
N	++	-	-	-	-	-	-	-	++	-	-	-
O[c]	-	++	-	++	-	+	+	+	-	++	++	++
P	-	++	-	++	-	+	-	+	-	-	-	-
Q	-	-	-	-	-	-	-	-	-	++	-	-
R	-	-	+	+	-	-	-	-	-	-	-	-
S	-	-	-	-	-	-	-	-	-	+++	-	-
T	-	-	-	-	-	-	-	-	-	+++	-	-

[a] culture medium used for incubation; -, not observed; +, represents 10 to 50 observable VLPs; ++, from 50 to 100 VLPs; +++, from 100 to 250 VLPs; ++++, 250 to 500 VLPs
[b] Namib desert site
[c] three most frequently observed phage morphotypes

plate and terminal fibers. The capsids exhibited icosahedral symmetry and appeared hexagonal in outline. Particle sizes ranged from head dimensions of between 50 nm and 125 nm in diameter, with tails of 100 nm to 300 nm in length. The most frequently observed VLP morphotypes were found to be two different types of *Myoviridae*, one with a head of approximately 75 nm, a thin neck, and a long, curved apparently-contractile tail of over 200 nm in length terminating in a complex base-like structure with numerous fibrils (Fig. 2K). The other common *Myoviridae* VLP resembled a PBSX-like phage (Wood *et al.*, 1990) with a head of approximately 50 nm, a thin neck, and a contractile tail of over 150 nm in length (Fig. 2L). These two VLP morphotypes were identified in both of the extraction media, with or without the addition of mitomycin C.

The *Siphoviridae*-like bacteriophage particle types were composed of non-contractile tails attached to non-enveloped icosahedral capsids. The shortest tail among the *Siphoviridae*-like morphotypes was 150 nm in length, terminating in an unusual ribbon-like structure but with an icosahedral capsid 50 nm in diameter (Fig. 2C) while the other Siphoviridae-like phage particle types had capsids ranging in size from 50∼100 nm in diameter with tails ranging in length from 150∼200 nm (Fig. 2A∼G).

Bacteriophage genome size diversity

To verify the potential viability of the extracted VLPs and characterize their genome diversity, we isolated the nucleic acids from our VLP preparations and separated them by pulsed field gel electrophoresis (Fig. 3). The sizes and relative abundance of the observable phage genomes were similar, though not identical, among the three sites sampled from the Namib desert. The diversity of genome sizes from sites A and B appeared more similar to each other than to site C, though the absolute amount of recovered VLPs and genomes from site B was less than those from sites A and C. Moreover, even within each of the three sites, different sized genomes were discernible after sand extraction in the different media and in the absence versus the presence of mitomycin C. However, as previously described (Prigent *et al.*, 2005), there did not appear to be an absolute correspondence between the genome sizes observed after pulsed field gel electrophoresis and VLP morphotypes discerned by electron microscopy.

All of the sites contained a DNA of approximately 12 kb in length, suggestive of a small Podoviridae-like phage. However, no bacteriophage-like particle of this size or morphotype was observed in any of the preparations. The majority of the genomes appeared to be 55∼65 kb in length, except when 1/4 TS media was used in samples from sites A and B. No large (>200 kb) phage genomes were detectable at site C, while site A yielded a DNA band of approximately 200 kb in length, but only when the sand was incubated in 1/4 TS media. Site B of the Namib desert produced the largest

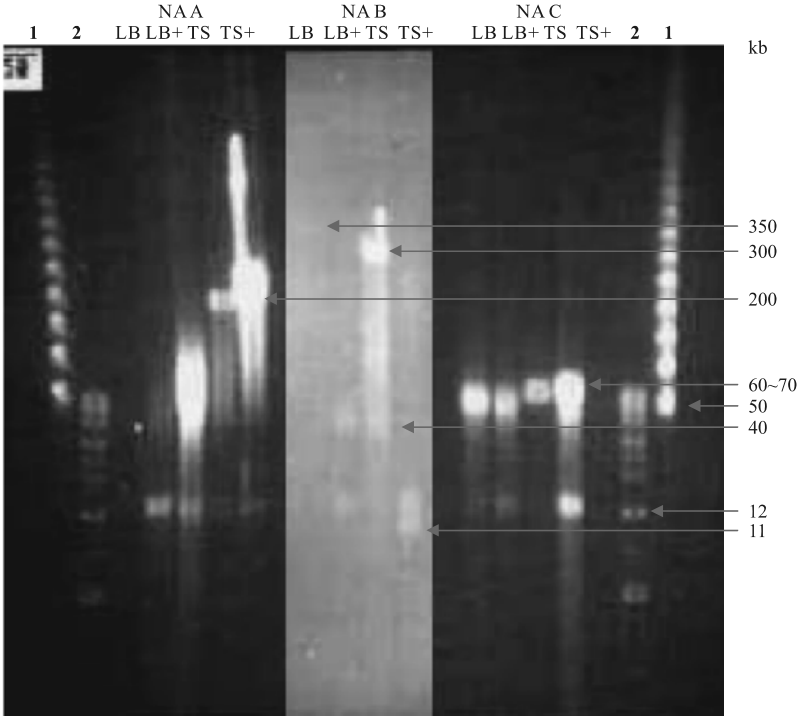

Fig. 3. Pulsed-field gel of phage DNA genomes from the Namib desert samples. The nucleic acids were extracted from the partially purified phages as described in the 'Materials and Methods' section and subjected to pulsed field gel electrophoresis through a 1% agarose gel using a Bio-Rad CHEF DR II apparatus. The gels were stained with ethidium bromide and photographed under UV light. Lanes 1 and 2; Pulsed field gel marker 50∼1,000 kb (Sigma-Aldrich) and l Mix 19 marker 8,271∼48,502 bp (Fermentas), respectively. Lanes NA A, NA B, and NA C represent nucleic acids isolated from partially purified phages after incubation of the Namib desert sand samples (A, B, C) in 1/5 LB or 1/4 TS media. + or -, with or without mitomycin C treatment.

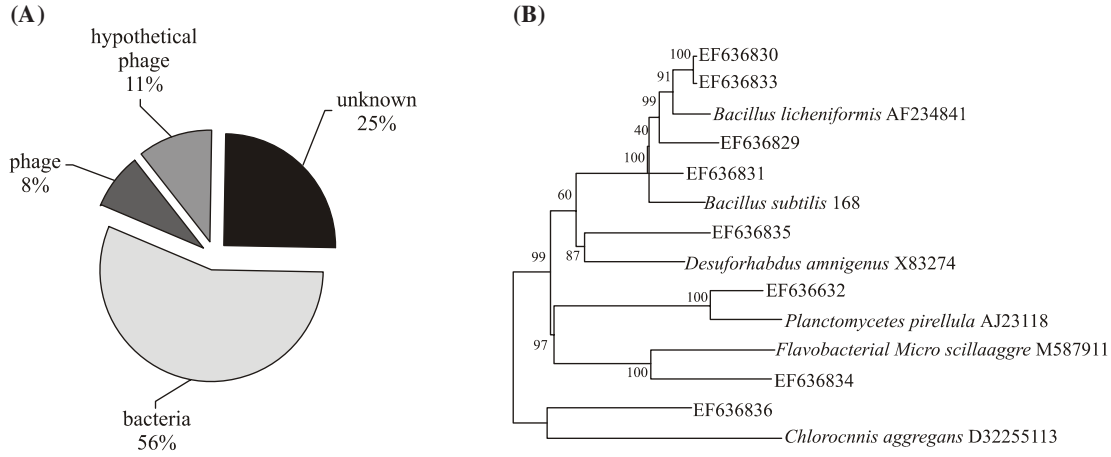

Fig. 4. Categorization of DNA sequences from the phage genomic and bacterial 16S rDNA libraries. (A) Phage DNA was extracted, amplified by PCR, cloned, sequenced, and analysed as described in the 'Materials and Methods'. DNA sequences were then categorised as being of bacterial, phage, putative phage or unknown origin. (B) Categorization of DNA sequences from the bacterial 16S rDNA sequences. The 16S rDNA was amplified by PCR from total extracted DNA, cloned, sequenced and analysed as described in the 'Materials and Methods'. The sequences were inserted in the multiple alignment window of the MEGA3 program and aligned via the program CLUSTAL W with Neighbour Joining using 1,000 as the Bootstrap value.

sized DNA bands, approximately 300 and 350 kb in length. Taken together, these results are consistent with the occurrence of intact phage particles observed by electron microscopy and suggest the existence of several full-length bacteriophage genomes in our preparations.

Bacteriophage and bacterial sequence diversity

Cloned libraries were prepared from the phage DNA genomes from the three sites of the Namib desert. Sequencing of randomly-selected DNA clones from the libraries revealed that approximately 50% of these sequences appeared to be of bacterial origin (Fig. 4A). Approximately one-half of these DNA sequences displayed significant homology to sequences from the genus *Bacillus*. In this regard, it is interesting to note that members of the *Bacilli* were abundant in the bacterial population of the sand samples from the Namib desert (see below). Of the remaining DNA sequences, approximately one-half had no significant match to any sequence in the database, even when the similarity level was raised to an exponent of 10^{-1} using TBLASTX. The remainder of the DNA sequences displayed generally low levels of homology, often to *Siphoviridae* phages of Gram-positive bacteria.

In order to begin to correlate phage and their potential bacterial hosts present in the sand, total DNA was extracted from the three sites. The 16S rDNA genes of eubacteria were amplified by PCR, the 1,500 bp DNA fragments were cloned, and several randomly-selected clones completely sequenced on both strands to determine if certain groups were predominant. Most of the 16S rDNA sequences did display significant (94~98%) homology to members of the Firmicutes, and in particular to members of the genus *Bacillus* (Fig. 4B). In addition, we also observed members of the Bacteroidetes, Planctomycetes, Chloroflexi, and δ-Proteobacteria (Fig. 4B). Members of the Bacteroidetes and Planctomycetes have been previously found to be present in desert soils (Dunbar *et al*., 2002; Chanal *et al.*, 2006), while some members of the δ-Proteobacteria can form spores within fruiting bodies.

Discussion

Bacteriophages are being increasingly recognized for their significant role in microbial ecology (reviewed in Fuhrman, 1999; Wommack and Colwell, 2000; Rohwer, 2003). The viruses of Archaea have also been extensively studied and have generally been isolated from the same extreme environments as their hosts (Forterre, 2001; Geslin *et al.*, 2003; Prangishvili and Garrett, 2005). The morphologies of Archaeophages have been found to be, in many cases, markedly different from those of bacteria (Prangishvili *et al.*, 2006). It has been demonstrated that bacteriophages can survive in extreme aquatic thermal environments, such as hot springs (Peng *et al*., 2001; Breitbart *et al*., 2004), and that adsorption to clay minerals can protect phage PBS1 from inactivation by ultraviolet irradiation (Vettori *et al.*, 1999). Moreover, their bacterial hosts have been shown to be protected from the effects of UV irradiation by the exopolysaccharides commonly found to surround cells within a biofilm (Elasri and Miller, 1999), suggesting that some phages and their hosts have evolved strategies to survive in environments previously believed inhospitable.

The efficacy of phage recovery from environmental solid matrices, such as soils and sediments, has begun to be examined (Rice *et al*., 2001; Breitbart and Rohwer, 2005; Williamson *et al.*, 2005). However, their presence in arid terrestrial environments, such as the surface sands of the Sahara desert and Arctic sea ice, has only recently been demonstrated (Borriss *et al*., 2003; Prigent *et al.*, 2005). Direct extraction of phages from the Namib sand did not allow the observation of VLPs by electron microscopy nor the visualization of distinct DNA bands on agarose gels (data not shown; Prigent *et al.*, 2005). This result may be due to a paucity of extracellular virions and/or to the diffi-

culty of extraction of intact virions from the sand. However, in addition to free particles (or virions), phages can exist as prophages (present as extrachromosomal plasmids or integrated within the genome of their hosts), or as virus particles in the cytoplasm of "pseudolysogenic" (also called "carrier state") bacteria (Ripp and Miller, 1998; Khemayan *et al.*, 2006; Williamson *et al.*, 2007). Thus, we introduced incubation steps in two different types of culture media (1/5 LB or 1/4 TS media) diluted to minimize nutrient shock to the endogenous bacteria (Reasoner and Geldreich, 1985) and in the absence and presence of mitomycin C, a mutagenic agent known to induce the lytic growth of some prophages (Canchaya *et al.*, 2003), to allow for phages within pseudolysogenic bacteria, plus SOS-inducible temperate phages, to complete a lytic cycle and be released from the cell (Williamson *et al.*, 2003; Prigent *et al.*, 2005; Williamson *et al.*, 2005). Using this extraction protocol, we recovered and observed a variety of virus-like particles (VLPs) from the sand of the Namib desert.

Under electron microscopy, the phage particle morphologies corresponded to the *Myoviridae* and *Siphoviridae* families of tailed bacteriophages. It is interesting to note that we observed a diversity of *Myoviridae*-type bacteriophages, whose complex contractile tails with potentially fragile caudal fibers might not be expected to be well adapted to such a severe habitat. However, we did not detect any members of the *Podoviridae* family, though members of this group were observed in samples from the Sahara desert (Prigent *et al.*, 2005).

The purification of phage nucleic acids and their separation using pulsed field gel electrophoresis revealed a number of potential bacteriophage genome DNA bands. The high molecular weight (greater than 150 kb) nucleic acid bands probably correspond to *Myoviridae*-type bacteriophages, known to have relatively large genomes (Ackermann and DuBow, 1987; Ackermann and DuBow, 2000). *Siphoviridae,* which have tails of lesser apparent complexity than Myoviridae-type phages, generally have genomes of less than 100 kb and would likely correspond to the lower molecular weight bands observed (Ackermann and DuBow, 1987; Ackermann and DuBow, 2000). We did not observe any large genomes (greater than 150 kb) in the southernmost sample (site C), but all samples produced a greater abundance and diversity of phage genomes after relatively short incubations in the presence of mitomycin C, suggesting that many active phages are present as SOS-inducible prophages. In this regard, it is important to note that the dry environmental conditions of these putative lysogens either precludes their induction by UV irradiation (Elasri and Miller, 1999; Elasri *et al.*, 2000) and/or supports the idea that bacterial exopolysaccharides can act as a protective barrier to the damaging effects of UV irradiation. It is possible, however, that some of the phage particle types have identical genome sizes, despite a different apparent morphology.

A genomic DNA library was prepared from a pool of the VLP preparations from the three different sites, and an initial analysis of Namib desert phage diversity was begun through sample DNA sequencing of randomly-selected clones. The relatively high proportion of apparent bacterial DNA sequences that we observed could be due to bacterial DNA contamination of our partially purified phage preparations and/or the presence of phage transducing particles. However, these sequences are also likely to include a significant number of phage genes that were annotated as being bacterial chromosomal sequences because the presence of a prophage and/or cryptic prophage was not recognized (Fouts, 2006). In addition, some bacteriophage genes can be highly homologous to their bacterial counterparts, and in the absence of a significant number of phage sequences for any given particular function, will appear to be of bacterial origin.

The limited amount of phage diversity examined to date could explain the low homology scores observed for many of our sequences to phage DNAs in the databases. The assignment of our sequences to specific categories of phages may also be problematic due to the presence of gene "modules" within phage genomes (Lucchini *et al*., 1999; Romero *et al.*, 2004) and the mosaic composition of some phage chromosomes (Canchaya *et al.*, 2003; Kwan *et al.*, 2005). Nonetheless, the phage-homologous DNA sequences we identified suggest a diversity of phages capable of survival in the Namib desert. In particular, we found that many phage sequences were homologous to *Siphoviridae* of Gram-positive bacteria. It is interesting to note that we found members of the *Bacilli* to be present in our sand samples by sequencing of amplified 16S rDNA genes. It is thus possible that Firmicutes and their phages represent a dominant population of the Eubacteria in the Namib desert.

In conclusion, we were able to establish the presence of bacteria and bacteriophage VLPs on the surface sands of the Namib desert. Further studies will allow a more in-depth examination of the phage and bacterial populations of the Namib (and other) desert and a characterization of their potential mechanism(s) of survival.

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